Amendments to the Specification

1. Please amend the paragraph starting on page 4, line 23, as follows.

~~Figure 1~~ Figure 1A-1D is a series of bar graphs showing the effects of antibodies directed against SUAM (A and B) and pepSUAM (C and D) on adherence and internalization of *S. uberis* into bovine mammary epithelial cells.

2. Please amend the paragraph starting on page 16, line 22, as follows.

Biotin-avidin-based binding assay (BABA) and ELISA-based binding assay were carried out on immobilized *S. uberis* microplates. LF from bovine milk and transferrin (TF) from bovine plasma were biotinylated. For the BABA assay, serial 2-fold dilutions of biotinylated LF were added into microplate wells, incubated, washed, and probed with ~~HRP-NeutrAvidin~~ HRP-NEUTRAVIDIN. The ELISA-based assay was essentially the same as BABA except that serial 2-fold dilutions of unlabelled LF were substituted for biotinylated LF. Rabbit anti-bovine LF antibody and HRP-conjugated donkey anti-rabbit IgG antibody were used as probes. Inhibition of LF-binding by unlabelled LF, TF, mannose, galactose, and lactose were also tested using BABA and ELISA.

3. Please amend the paragraph starting on page 24, line 1, as follows.

Extracted bacterial surface proteins (10 Fg/lane) were electrophoresed on 10% SDS-PAGE. Gels were stained with Coomassie brilliant blue or transferred onto nitrocellulose membrane using Trans-Blot SD Semi-Dry Electrophoretic Transfer Cell (Bio-Rad). Unbound

sites on blots were blocked with 3% casitone. Blots were probed with LF (ICN, 5 Fg/ml) in PBS ~~Tween 20~~ TWEEN 20 (PBST) containing 0.1% casitone for 6 h at 4°C, followed by four washes with PBST. Procedures for further probing of blots with rabbit anti-bovine LF antibody and HRP-conjugated donkey anti-rabbit IgG antibody were as described previously (Fang and Oliver, 1999). Blots without probing with LF and rabbit anti-bovine LF antibody were included as negative controls.

4. Please amend the paragraph starting on page 25, line 14, as follows.

Thirty ml of PBS (pH 7.4) containing 30 mg of SDS-extracted *S. uberis* surface proteins were loaded into a bovine LF-coupled CNBr-activated ~~Sepharose~~ SEPHAROSE 4B column. SDS-extracted surface proteins were incubated with shaking for 2 h at 4°C with 7 ml of ~~Sepharose~~ SEPHAROSE 4B covalently linked to bovine LF (ICN, 21.4% iron saturation and 97.5% protein content). The LF-~~Sepharose~~ SEPHAROSE 4B slurry was loaded into a chromatography column (1.25 cm x 9 cm; total volume 70 ml) (Pfizer, New York, NY). The column was subsequently washed with 10 volumes of TBS (50 mM ~~Tris-HCl~~ TRIS-HCl (pH 7.4) + 150 mM NaCl containing 0.1% ~~Triton-X~~ TRITON X 100) to remove nonspecific-binding proteins using a peristaltic pump at a flow rate of 1 ml/min until absorbance at 280 nm approached zero. The column was eluted with a sodium chloride gradient from 0.1 M to 1 M NaCl in TBS. Fractions (10ml/fraction) were analyzed by absorbance at 280 nm, SDS-PAGE and Western blot using rabbit anti-bovine LF antibodies and biotinylated LF as probes. Fractions containing SUAM were pooled, dialyzed against PBS and stored at -70°C until use.

5. Please amend the paragraph starting on page 26, line 9, as follows.

Analysis of fractions revealed the presence of a protein in fraction number 14 to 32 eluted at 0.5M NaCl. The molecular mass was estimated to be ~ 112 kDa using ~~Gel Scan~~ GEL SCAN (Corbett Research, Mortlake, NSW, Australia). Results from SDS-PAGE and Western blot analysis indicated that this band had LF-binding affinity. The yield of purified SUAM was 20 Fg/ml (total 10 ml) from 3 liters of THB-grown *S. uberis*.

6. Please amend the paragraph starting on page 26, line 17, as follows.

Excised PVDF membrane (PerkinElmer Life and Analytical Sciences, Inc., Boston, MA) containing ~112 kDa SUAM band was analyzed. The protein was sequenced on an Applied Biosystems model 477A sequencer (Applied Biosystems, Foster City, CA) equipped with on-line PTH analysis using the regular program O3RPTH. The PTH-derivatives were separated by reverse-phase HPLC over a ~~Brownlee~~ BROWNLEE C-18 column (220 x 2.1mm). The initial yield for the coupling step was calculated from the amount of PTH-derivatives present in the first cycle and by the amount of protein spotted. As a standard marker for amino acid sequence, the repetitive yield from myoglobin was determined from peak heights of valine, leucine, and glutamic acid according to the positions. The repetitive yield from β-lactoglobulin was calculated for leucine, isoleucine, and valine residues. The N-terminal amino acid sequence of SUAM was D D M T T A D Q S P K L Q G E E A (T/A) L (I/A) (V/K) (Seq. ID No. 12).

7. Please amend the paragraph starting on page 30, line 7, as follows.

To ensure that SUAM is not a rare protein found only in one strain of *S. uberis*, and that research or prophylactic products developed will have broad significance, several strains of *S. uberis* from diverse locations were tested by Western blotting. Strains evaluated were from Tennessee, Colorado, Washington and New Zealand. The different *S. uberis* strains were cultured overnight in Todd Hewitt broth and surface proteins were extracted in Laemmli sample buffer. SDS-PAGE polyacrylamide gels (7.5%) were electrophoresed followed by transfer to nitrocellulose membranes. They were blocked in PBSTG (phosphate buffered saline, 0.05% (v/v) TWEEN-20 ~~Tween-20~~, and 0.1% (w/v) porcine gelatin) for 1 h. Affinity purified rabbit anti-pepSUAM and rabbit anti-SUAM antibodies were diluted in PBSTG (1:2000) and blots treated for 1.5 h. Following washing of blots with several changes of PBST, a 1:2000 dilution in PBSTG of peroxidase-conjugated affipure F(ab')2 fragment donkey anti-rabbit IgG (H+L) was applied. The SUAM protein band was revealed with the peroxidase substrate 4CN (4-chloro-1-naphthol). The presence of a single dominant band on a blot of total *S. uberis* detergent extracted surface proteins attests to the specificity of the antibodies. The 112 kDa SUAM protein band is clearly visible. These results establish that SUAM is a ubiquitous protein in *S. uberis* strains and that pepSUAM may play a role as a universal immunogen to protect against *S. uberis* mastitis.

8. Please amend the paragraph starting on page 31, line 12, as follows.

Cross-reactivity of rabbit anti-SUAM whole protein antibodies and rabbit anti-pepSUAM antibodies between different *Streptococcus* species was investigated. Strains of *S. dysgalactiae* subsp. *dysgalactiae*, *S. agalactiae* (from animals and humans), and *Streptococcus pyogenes* were

cultured overnight in Todd Hewitt broth and bacterial surface proteins were extracted in Laemmli sample buffer. SDS-PAGE polyacrylamide gels (7.5%) were electrophoresed followed by transfer to nitrocellulose membranes. They were blocked in PBSTG (phosphate buffered saline, 0.05% (v/v) TWEEN-20 ~~Tween-20~~, and 0.1% (w/v) porcine gelatin) for 1 h. Affinity purified rabbit anti-pepSUAM and rabbit anti-SUAM antibodies were diluted in PBSTG (1:2000) and blots treated for 1.5 h. The next treatment after washing blots with several changes of PBST was a 1:2000 dilution in PBSTG of peroxidase-conjugated affipure F (ab') 2 fragment donkey anti-rabbit IgG (H+L). The SUAM protein band was revealed with the peroxidase substrate 4CN (4-chloro-1-naphthol). Western blot results showed cross reaction of pepSUAM and SUAM antibodies with proteins of other *Streptococcus* species, including the human pathogen *S. pyogenes.* The cross reaction with other proteins or protein fragments indicates that SUAM and its functions are conserved or partially conserved between Streptococcus species and that a vaccine based upon SUAM would have broad application.

9. Please amend the paragraph starting on page 16, line 7, as follows.

Experiments were conducted to examine binding of lactoferrin (LF) by strains of *S. uberis* causing bovine mastitis and to identify proteins from the bacteria involved in LF-binding. Four strains of *S. uberis* isolated originally from dairy cows with mastitis and *S. uberis* ATCC13387 (American Type Culture Collection, ~~Rockville, MD~~ Manassas, VA) were evaluated. After growth, bacterial cultures were washed and split into two equal portions: one for incubation in milk and the other in phosphate buffered saline (PBS) (as controls). Bacterial surface proteins from pellets were extracted using 0.2% sodium dodecyl sulfate (SDS) and electrophoresed. Gels

were silver-stained or transferred onto nitrocellulose membranes for immunoblotting using rabbit anti-bovine LF antibody and HRP (horseradish peroxidase)-conjugated donkey anti-rabbit IgG antibody as probes.